EFFECT OF ULTRASOUND ON ANAEROBIC FERMENTATION BY *SACCHAROMYCES CEREVISIAE*

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Thesis submitted to the Faculty of Chemical and Natural Resource Engineering in fulfillment of the requirements for the award of the Degree of Bachelor of Chemical Engineering

Faculty of Chemical and Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

FEBRUARY 2013

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LIST OF ABBREVIATIONS AND SYMBOLS

A	Area
P	Power
°C	Degree Celcius
mL	Millileter
g/L	Gram per militer
gL^{-1}	Gram per liter
h	Hour
s	Second
W	Watt
$W\ cm^{-2}$	Watt per Centimeter Squared
%	Percentage
m	Meter
µ	Micro
w/w	Weight per weight

KESAN ULTRASOUND TERHADAP PENAPAIAN ANAEROBIK OLEH *SACCHAROMYCES CEREVISIAE*

ABSTRAK

Kajian ini menerangkan tentang kesan ultrasound terhadap penapaian anaerobik *Saccharomyces cerevisiae*. Pengeluaran biojisim yang kurang dan hasil etanol yang lebih tinggi dalam penghasilan etanol menyebabkan ultrasound diperkenalkan dalam proses ini. Pengasilan etanol yang tinggi telah menghalang penapaian dan memperlahankan pertumbuhan biojisim. Kaedah ultrasound digunakan untuk menentukan hasil pengeluaran etanol, pertumbuhan biojisim dan untuk menentukan parameter kinetik. Penapaian telah dijalankan dengan menggunakan ultrasound dan tanpa ultrasound yang digunakan sebagai kawalan. Penapaian telah dijalankan selama 24 jam dan setiap 2 jam sampel diambil. Kemudian sampel yang telah diambil dikenakan kepada ultrasound dengan dua kitar tugas yang berbeza iaitu 10% dan 30% kitaran. Kepekatan biojisim, kepekatan glukosa, kepekatan etanol telah diperolehi daripada kajian ini. Parameter kinetik juga dapat ditentukan. Daripada kajaian ini, rejimen sonication yang terbaik adalah 10% kitaran berbanding 30% kitaran. Nadi ultrasound telah memberi kesan kepada pertumbuhan biojisim yang berkaitan dengan bilangan pertumbuhan sel yang berjaya. Apabila nadi ultrasound rendah, pertumbuhan biojisim meningkat, serta kepekatan etanol juga meningkat. Hasil etanol terakhir adalah $12.64 gL^{-1}$. Kajian ini perlu diteruskan lagi dengan menggunakan komposisi yang berbeza untuk glukosa dan jenis yis atau kulat yang berbeza. Selain itu, kajian ini juga boleh diteruskan dengan menggunakan kuasa ultrasound yang berbeza atau intensiti yang berbeza untuk menentukan kesan ultrsound terhadap penapaian anaerobik.

EFFECT OF ULTRASOUND ON ANAEROBIC FERMENTATION BY *SACCHAROMYCES CEREVISIAE*

ABSTRACT

This research describe about the study on the effect of ultrasound on anaerobic fermentation of *saccharomyces cerevisiae*. The less biomass production and higher ethanol produce in the production of ethanol causes the ultrasound to apply for this process. The high ethanol inhibits the fermentation and slowed the biomass growth. The ultrasound method is applied to determine the production yield of ethanol, the growth of biomass and to determine the kinetic parameter. The fermentation was conducted with the ultrasound and without the ultrasound which is used as a control. The fermentation was conducted for 24 hour and every 2 hour the sample was collected. Then the sample was attached to the ultrasound with two different duty cycles which is 10% and 30% duty cycle. After that, the sample was analysed. The biomass concentration, glucose concentration, ethanol concentration was obtained from this research. The kinetic parameter also was determined. From this research, the best sonication regiments are 10% duty cycle rather than 30% duty cycle. The pulse ultrasound effect the biomass growth which is related to the number of successive growth of cell. The lower the pulse ultrasound, the biomass growth increase, as well as the ethanol concentration also increase. The final ethanol yield is $12.64gL^{-1}$. This research is reaccommodated to use a different composition of glucose and different type of yeast or fungus. Besides, use a different power or different sonication intensity to determine the effect of ultrasound towards the fermentation.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Recently, fermentation is widely used in order to produce a wine. Product from the fermentation is in form of alcohol. In wine fermentation, the *Saccharomyces cerevisiae* play an important role which is to change the grape sugar into the ethanol, (Parapouli, 2010). *Saccharomyces cerevisiae* can growth well in aerobic and anaerobic conditions. Aerobic fermentation is condition which is oxygen is supplied to the fermentation. Meanwhile, an anaerobic fermentation is the condition in which oxygen is absent or not present and other gas is used such as nitrogen.

Some of studies use an ultrasound effect to the fermentation is reported. A study carried out by Sulaiman et al., (2011) state that ultrasound had been used in laboratory scale for ruptured the cell walls in order to release intracellular products. Ultrasound also related with the damage to cell. Ultrasound wave could be divided into two powers which is high power and low power, (Jomdecha & Prateepasen, 2006). Both powers give a different effect towards the reaction or process.

1.2 Problem Statement

Increasing demand in alcohol and wine fermentation makes the production of ethanol also increase. In the production of ethanol, less biomass is produce while higher ethanol produces in anaerobic fermentation of *Saccharomyce cerevisiae*. Due to the high ethanol production, ethanol inhibits the fermentation reaction and slowed the biomass growth (cell). A study by Hoppe and Hansford (1982) stated that the ethanol inhibit the biomass yeast growth in anaerobic fermentation. Then the study is carried out in order to optimise the ethanol production by depend on the biomass growth. *Saccharomyce cerevisiae* yeast being used in production of ethanol because in previous study stated that, this yeast can generate and produce more ethanol than other type of yeast. The ultrasound is applied to increase the biomass growth.

1.3 Research Objective

The main objective of this research is to study the effect of ultrasound to anaerobic fermentation of *Saccharomyces cerevisiae.*

The measurable objective for this research are:

1.3.1 To examine the effect of sonication regiments on anaerobic fermentation of *Saccharomyces cerevisiae.*

1.3.2 To determine the kinetic parameters of the fermentation with and without ultrasound.

1.4 Research Questions/Hypothesis

1.4.1 What is the production yield of ethanol in anaerobic fermentation?

1.4.2 What is the effect of ultrasound on growth of Saccharomyces cerevisae in anaerobic fermentation?

1.4.3 What is the kinetic parameter of anaerobic fermentation of Saccharomyces cerevisae?

1.5 Scope of Study

In order to gain the objective, scope of study is needed as follows:

a) To study the effect of sonication regiments for improving productivity of a live cell in *Saccharomyces cerevisae* fermentation.

b) To determine the kinetic parameters of the fermentation with and without ultrasound.

1.6 Significance of Study

Ethanol fuel gives benefits toward human because it acts as a bioenergy. The ethanol production is having an environmental friendly because use a biological instead of using the chemical production. The ethanol also can serve as fuel to the mobile transportation. Beside that, ethanol will be used as a medical treatment to the patient. Ultrasound method will be used into the industry to enhance the bioprocess performance and to increasing the production of ethanol. Ultrasounds also can enhance the fermentation time. By introduce ultrasound to industry, the ethanol production will increase, and increasing the economy of industry in which the time of fermentation decrease so that it will save the cost for the production and raw materials.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

A literature review was introduced to classified studies that related to the topic. There are four themes in this literature review which is *saccharomyces cerevisiae* yeast, application of *saccharomyces cerevisiae* yeast, substrate**,** ultrasound, effect of ultrasound, application of ultrasound in fermentation, application of ultrasound in biotechnology, alcohol fermentation and anaerobic fermentation of *saccharomyces cerevisiae* yeast that have been studies in this chapter.

2.2 *Saccharomyces Cerevisiae* Yeast

Saccharomyces cerevisae is atype of yeast. Study by Dequina and Casaregol (2011) stated that there are eight of different type of this yeast that mostly valuable in food and beverage fermentation. For the type of yeast that related to antrophic environments is *Saccharomyces cerevisiae, Saccharomyces bayanus and Saccharomyces pastorianus.* Meanwhile, *Saccharomyces paradoxus, Saccharomyces kudriavzevii, Saccharomyces cariocanus, Saccharomyces mikatae* and the lately express *Saccharomyces arboricolus* are mainly isolated from natural environments.

Industrial of Saccharomyces yeasts are not commonly in the form of interspecific hybrids, (Dequina & Casaregol, 2011). The hybrid nature of these yeast genomes is an benefits that bring together the properties from each of the parental stains. Their features as hybrids, including type of species that given rises to them and the difficulty of their genomes, differences of their specialization and industrial environment (Figure 2.1).

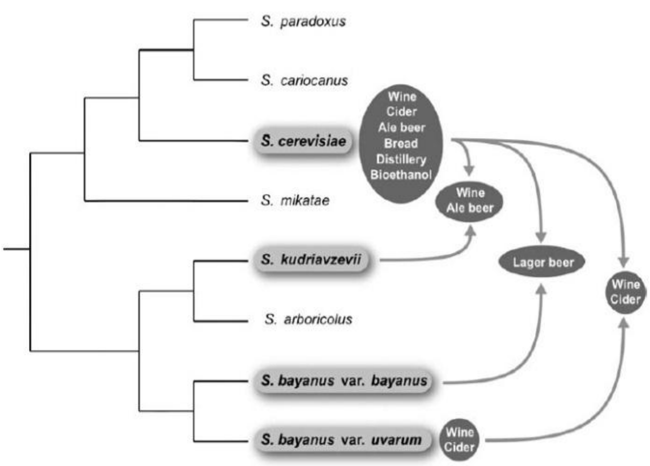

Figure 2.1 Phylogenetics relationship between saccharomyces species and their industrial application. Light grey for industial process or in hybrids. Dark grey for products of hybrids and non-hybrids.Arrows shown as hybrids.

(Source: Naumov, 1994)

Variety of mono-, di- and oligosaccharides and respiratory substrate can be used by *Saccharomyces cerevisae* such as ethanol, glycerol, acetic acid, lactate and pyruvate, (Papagianni, Boonpooh, Mattey & Kristiansen, 2007). Another study by Deken (1996) stated that *Saccharomyces cerevisae* is the type of prototypical Crab-tree-positive yeast for aerobic fermentation. Crab-effect is regulatory system which is including the repression of energy source by another type of energy source.

A study by Papagianni et al. (2007) is reported that *Sacchromyces Cerevisae* fermentated in anaerobic conditions and acts as a facultative anaerobe. *Sacchromyces Cerevisae* is able to respire or breathe on low concentration of sugar or in the respirator substrate. This yeast can growth on simple sugar which is glucose also in dissacharide sucrose, (Lin & Tanaka, 2006).

2.3 Application of *Saccharomyces Cerevisiae* Yeast

Saccharomyces cerevisiae yeast is applicable to fermentation in anaerobic growth, (Liden et al., 1995). Beside that, it also can display fermentative metabolism in aerobic culture state, (Cortassa & Aon, 1998). *Saccharomyces cerevisiae* is widely used in some industrial application in the production of beer, baker's yeast, alcohol, wine, heterologous proteins and ethanol fermentation, (Olsson & Nielsen, 2000). This is because it can manipulate growth and have relatively similar structure of human cells.

According to Parapouli (2010) the wine fermentation have been use a *Saccharomyces cerevisiae* yeast and it play most important role to changing the grape sugar into the ethanol. For wine industries, it utilizes a commercial yeast strains as a stater culture. *Saccharomyces cerevisiae* yeast also used in ethanol production on anaerobic fermentation by applied the ultrasound and without ultrasound in order to determine the differences of productivity between these two condition. A study by Sulaiman et al., (2011) has reported that this yeast is the only yeast that consider to some level in ultrasound fermentation. Besides that, the *Saccharomyce cerevisiae* yeast is used in fermentation because when the ultrasound is applied the yeast will assess to some level.

2.4 Substrate

Glucose is some type of the substrate that use in fermentation of *Saccharomyces Cerevisae*. According to Papagianni et al. (2007) the glucose is used as a carbon source in fermentation and as a suitable metabolism in EMP pathway which give a product of ethanol. Another type of sugar substrates to the fermentation of *Saccharomyces Cerevisae* is the α-glucoside which is including the maltose, sucrose and maltotriose, (Dequin, 2001). Maltose is chosen as the most use in the fermentation of *Saccharomyces Cerevisae*.

2.5 Ultrasound

Ultrasound is some kind of mechanical elastic energy or wave. The power of ultrasound is divided by two which is high power and low power, (Jomdecha & Prateepasen, 2006). Ultrasound has been use in research to produce, collect or measure sound wave in medium. Beside that, the effect to an irradiation medium also had been study by using ultrasound wave which is produce from ultrasound equipment. According to Lanchun et al., (2003) ultrasound has the transmission in material and the phenomena of calefaction and cavitation when it passes through in medium.

A cavitation phenomenon is a major mechanism that causing change of biological tissue which mainly increasing the membrane permeability during the fermentation process, (Bommannan et al., 1992). Membrane permeability is referring to the diffusion of molecule through the membrane. In this phenomenon, the bubbles will exits until it stable and then will collapse at critical state then produce high temperature and pressure.

In addition, the ultrasound can enhance the yeast growth, (Matsuura, 1994). The mass transfer around the cell and inside the cell is increasing when ultrasound is applied in direction of diffusion. (Laugier, 2008). Low frequency level of ultrasound will speed up the movement of liquid medium, increase mass transfer and reaction rates for homogeneous and multiphase system, (Liu et al., 2006). Ekaterina (2009) stated that productivity of fermentation process also can be improve by applied the ultrasound towards to the process.

2.6 Effects of Ultrasound

Ultrasound gives an effect towards the fermentation and cell. Several studies reported the ultrasound give effect to the some factor. The factor is divided to three parts which is cavitation, mass transfer enhancement and thermal effect.

2.6.1 Cavitation

Cavitation is a process of a bubbles form when the ultrasound is being applied to the medium. Cavitations enhance the membrane permeability of the cell. There two type of cavitation which is non-internal and internal cavitation. Non-internal cavitation is a stable cavitation when bubble form with a constant number of acoustic cycle meanwhile internal cavitation is unstable when the bubbles oscillate on unstable cycle, (Riesz & Kondo, 1992). Figure 2 shows the bubble form when ultrasound is applied.

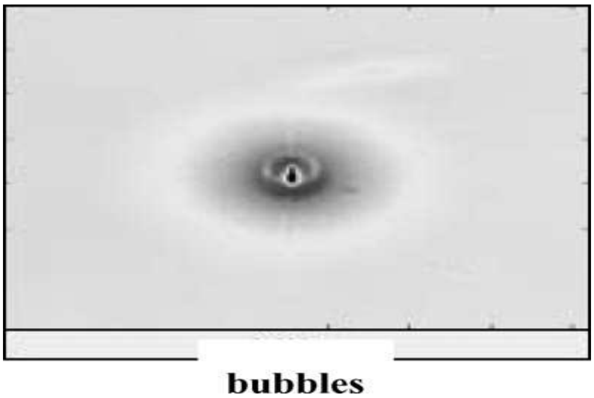

Figure 2.2 The formation of cavitation bubble induced by ultrasound,

(Source: Liu et al., 2006)

2.6.2 Mass Transfer Enhancement

Study by Bar (1988) stated that mass transfer and reaction rate increasing when the low intensity of ultrasound is applied. The gas bubbles generate along the circulation liquid was known as microstreaming. Microstreaming will lead the flux of reagents to the cell and thus increasing the reaction rate. Ultrasound also increases the mass transfer for both artificial and biological membrane, (Liu et al., 2006).

2.6.3 Thermal Effect

The use of ultrasound to the medium will increase the temperature of the medium when the medium absorb the energy from the ultrasound. This phenomenon does not make as a main activation of the fermentation. But can use a important part or factor to the fermentation, (Liu et al., 2006).

2.7 Application of Ultrasound in Fermentation

Applications of ultrasound towards fermentation process have been widely use in food industry and alcohol fermentation nowadays. Study by Lamberti et al., (2009) uses an ultrasound to monitor the wine alcohol fermentation. The ultrasound also been applied in biorenewables as a new concept and have a function to improve the enzymatic hydrolysis and consequent to the ethanol fermentation. Ultrasonic is use in the process to produce and determine the sound wave in medium of cells then study it characteristic and properties. In addition, ultrasonic wave is being applied to investigate the energy effect to an irradiation medium from the generated ultrasonic equipment, (Jomdecha & Prateepasen, 2006).

Jomdecha and Prateepasen (2006) said that the power of ultrasound have some applications. For high power ultrasonic involve in cleaning, welding and biological or chemical process while low power used for testing a material, measuring and communications are depend to the properties or character of the received ultrasonic waves. Beside that, the ultrasound range about 1-10MHz is used in medical imaging, (Chisti, 2003). High power in ultrasound treatment for aqueous media has been used to reduce hatch times offish eggs and germination times of seeds. In one study, intermittent ultrasound treatment for accumulative period of 150 s using a 25 kHz tube resonator (constant 80 W effective output) caused 76% increase in the release of intracellular gentamicin during production by *Micromonospora echinospora.*

A study by Lamberti et al., (2009) said that an ultrasonic method is used to investigate the alcohol and extract contains in wines, even though they not have go through enough in fermentation process itself, an relationship between ultrasonic parameters and the concentrations of alcohol and soluble solids was proposed, indicating that these concentrations mainly determine the speed of the sound in fermenting media.

2.8 Application of Ultrasound in Biotechnology

The varieties of method have been researched to improve the biotechnology process. Ultrasound is the new method to improve the bioprocess performance. This ultrasound method can reduce the process time compare to the conventional method. For biotechnology, ultrasound is use in the production of biofuel from triglycerides, (Rokhina, 2009). The ultrasound will used to optimize the change of triglycerides to biofuel. The low frequency of ultrasound (28 and 40 kHz) will give influence towards the production of biofuel from triglycerides.

Ultrasound method also used in enzyme biocatalysts process. For the last few years, ultrasound has been used to improve or increase the enzyme-catalyzed waste treatment, (Rokhina, 2009). Biosensor also used ultrasound to aggregate and drive in order to enhance the sensitivity and efficiency of biosensors, (Zourob, 2005).

2.9 Alcohol Fermentation

In fermentation, the energy is required to produce the ethanol. This energy is needed for the growth of yeast cell. Glucose acts as a carbon sources then will produce an energy to the fermentation process. The chemical equation when the alcohol fermentation occurs is illustrated in Figure 2. Based on this reaction, the theoretical yield of ethanol will produce is 0.511 and for carbon dioxide is 0.489 on a mass basis of glucose metabolized, (Bai et al., 2008).

$$\text{Glucose (carbon source)} \longrightarrow \text{2 ethanol + 2 carbon dioxide} \qquad (2.1)$$

$$C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2 \qquad (2.2)$$

Figure 2.3 Chemical reaction of ethanol production,

(Source : Teh & Lutz, 2010)

2.10 Anaerobic Fermentation of *Saccharomyces Cerevisiae* Yeast

According to the Dake et. al (2010) common definition for the fermentation in chemical term is the conversion of carbohydrate to the acids or alcohols. Another definition is the breakdown of carbohydrates to ethanol, carbon dioxide and water by using micoorganism, (Osunkoya & Okwudinka, 2011). Meanwhile, anaerobic fermentation is the fermentation in absence of oxygen and nitrogen is being used instead off oxygen. For *Saccharomyces cerevisiae* yeast can growth well under the aerobic and anaerobic conditions. Aerobic condition is in the present of oxygen.

A study by Brandberg, Gustafsson & Franzen (2007) reported that the limitation of nitrogen can cause a biosynthesis to be reduced. Complete starvation for nitrogen give many benefit towards *Saccharomyces cerevisiae*. A change to the macromolecular composition was occurred and transfer of glucose also will inactivate. Nitrogen will acts as a limiting factor for cell growth when it is being suppling continuously.

CHAPTER 3

METHODOLOGY

3.1 Introduction

Fermentation is carried out in order to determine the effect of sonication. The fermentation converted the glucose into the ethanol by using yeast *saccharomyces cerevisiae*. The fermentation was accomplished with and without sonication. The step involve to do this research is micoorganism, preparation of agar plate, culture, preparation of inocolumn, fermentation process and ultrasound procedure. There three step to analysis this research which is ethanol concentration analysis, biomass concentration and glucose concentration.

3.2 Micoorganism

Baker's yeast *(Saccharomyces cerevisiae)* was obtained from the University Malaysia Pahang. The yeast was provided in the form of solid.

3.3 Preparation of Agar Plate

The maintenance agar medium was prepared by mixing with deionized water and the composition of 50gL^{-1} glucose, 2gL^{-1} yeast extract, 6.25gL^{-1} $(NH_4)_2SO_4$, 2gL^{-1} $MgSO_4.7H_2O$, 4 gL^{-1} KH_2PO_4 and 15gL^{-1} agar and stir using magnetic stirrer with the heat on and poured into the 1L Schotte bottle. After that, the solution was sterile by autoclaving at 121°C for 20 min. The solution are left to cool at 50°C after sterilize. About 15-20ml of the sterilize solution per plate was poured into the plate under laminar flow hood then left it to cool to solidify (agar plate) and keep it about 4°C in chiller until it use.

3.4 Culture

One scoop of solid yeast *Saccharomyces cerevisiae* was poured in sterile medium with previous composition without the agar and incubates in orbital shaking incubator at 30°C and 180rpm for 24 hour and then inoculated the cell to the agar plate. The agar plates then incubate in incubator at 30°C for 24hr. After the